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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 12 MAY 1954 No. 5

EARLY EXPERIENCES IN PROTEIN NUTRITION—A RETROSPECT

Taking my degree with Professor E. G. Conklin at Pennsylvania in zoology (1901), my thesis was entitled "Absorption and Secretion in the Digestive System of the Land Isopods." These crustacea, commonly called "sow bugs," often live under the boards of a damp cellar or under the rotting bark of fallen trees. My chief and several other American and European zoologists had noted the relatively enormous cells lining the tubular intestine, and had observed cytologic signs of absorptive activity of the cells.

Professor Conklin had removed the intestinal tract and, after fixing and staining the cells, thought he had observed ameboid processes from the large nuclei extending toward the lumenal border of the cells, "as if searching for food." Conklin turned over this problem to me, to find out whether food could be identified in the absorbing cells.

Fallen trees were plentiful in Woodland Cemetery back of the laboratory and most of my specimens came from that source. The animals, measuring about 1 cm. in length, were kept in small dishes about 2 inches in diameter with damp filter paper under the cover, because the gills would not function without moisture. It was necessary to wait a day or two for the intestine to become at least half freed of bits of dead leaves, rotting bark, etc., so that one could feed the "bugs" on different kinds of more edible food. Individuals could be starved for a certain number of days, and when they had become tired of eating the filter paper, they would eat cereal breakfast foods of almost any description. Starches, however, are difficult to stain inside cells, so I resorted to several kinds of fats and proteins.

Starved for various lengths of time, animals were then allowed to eat raw beef for ten minutes, after which they were isolated and killed at successive intervals, and the intestines were fixed, stained and examined. Albumose granules often could be identified inside the cells. Absorption of fat was obtained by feeding the "bugs" with butter, beef suet, or olive oil. Not all of the fat was absorbed, for fecal pellets containing fat often were found forty-eight to seventy-two hours after feeding, but enough was absorbed to identify fat globules.

Conklin agreed that the ameboid processes from the nuclei were not reaching for food, after we had injected fixing fluids into the lumen of the gut and found that they extended away from the lumen.

After teaching for two years at a small college in Pennsylvania, I joined Lusk's laboratory at University and Bellevue Medical College, New York City. When I reported for work in September 1903 Lusk suggested we investigate the sparing action of gelatin on the protein metabolism of dogs.

Carl Voit, in collaboration with Bischoff (T. L. W. Bischoff and Voit, "Die Gesetze der Ernährung des Fleischfressers," *C. F. Winter'sche, Leipzig (1860)*), had established experimentally the concept perceived by F. C. Donders ("Die Nahrungsstoffe," *p. 72 (1853)*) that gelatin reduces the protein requirements of the body. In 1872 (*Z. f. Biol.* **8,** *297, see esp. p. 344*) Voit declared that gelatin exercises its sparing power on protein both with large and small quantities of protein fed at the same time in much higher degree than either fat or carbohydrate.

We were to find out how much of the body's protein could be spared by gelatin. Holmes Jackson, then assistant professor of physiological chemistry, gave me instruction in the Kjeldahl procedure for determining

nitrogen in the excreta, and Lusk taught me how to catheterize the bladder of female dogs so as to get "every drop" of urine. Having been brought up in the rigid discipline of Voit's laboratory, Lusk warned me that I must not smoke a pipe while titrating the ammonia in the Kjeldahl receiving bottle. One day he caught me smoking and when I showed him that I could not get any change in the color of the alizarin indicator, even when I blew smoke into the receiver, he couldn't believe it. If Voit said smoke would ruin the titration, Lusk was bound to believe it, though neither of them, I think, had ever put it to the test.

That incident created a mild tension between Lusk and me for several weeks, and I requested that I be permitted to make my own mistakes and be responsible for the results. He assented. Conklin had believed in putting a student on his own, although he was helpful always when appealed to for assistance.

The gelatin feeding to dogs progressed satisfactorily until I came to the supplementation of gelatin with amino acids. I was trying to confirm an observation of M. Kauffman (*Arch. f. d. ges. Physiol.* **109,** *440 (1905)*) that the addition of tyrosine, cystine and tryptophan would restore gelatin to the full nutritive value of high class protein. A small amount of money from the slender budget of the department was available and I set out to find these three amino acids. I found all but tryptophan. Finally, an instructor named Emerson at the Massachusetts Institute of Technology undertook to make some of this acid for me.

It was in the summer of 1905, while I was translating R. Tigerstedt's authoritative "Lehrbuch der Physiologie des Kreislaufes" (*Veit, Leipzig (1893)*) that I heard from Emerson and he admitted he could not furnish me with more than 1.8 g., which he was sending. He said this quantity had cost him $25.00. The money was sent and then I undertook the feeding of the three amino acids with gelatin to white mice.

My family and a friend, typing for me, went to Collegeville, Pennsylvania, for the summer, where I had been "professor of biology and lecturer in chemistry!" in Ursinus College. I took the white mice with me, and cajoled the caretaker of the building into letting me use some small lockers, in the biology department, in which to house the mice. Unfortunately, the lockers with wood-paneled doors were not resistant to gnawing mice. The old dean and professor of Greek, who was watching my experiments carefully, was highly indignant when he saw some evidence of gnawing and had observed that the mice dropped little black pellets on the counters! The board of trustees acted promptly to the dean's complaint that I was despoiling the building and I was obliged to abandon my experiments. The expensive tryptophan was wasted.

To check the results on dogs I starved myself for three days, then ate oatmeal and beefsteak with butterfat and sugar for two days just as I had fed my dogs, and replaced a two-thirds portion of the meat with gelatin. The experiment came out very nearly the same in sparing effect as with the dogs.

My next project concerning proteins was the study of nitrogen balance during menstruation and pregnancy in the dog. A chance observation made in 1907, that the excretion of nitrogen in the urine of a fasting pregnant dog was more profoundly influenced by a small amount of carbohydrate than in the urine of a fasting nonpregnant animal, directed my attention to the quantitative and qualitative alterations in the metabolism produced by menstruation and pregnancy.

Before this I had measured the energy metabolism of a dog, which in two pregnancies had delivered only one pup in the first, and 5 pups in the second. It turned out that the energy production of the pregnancies at or near their culmination was proportional to the weights of the offspring delivered, thus, 46 calories for a 280 g. pup and 258 calories for 5 pups weighing 1560 g. Calculation of the energy production was by

indirect calorimetry, involving the nitrogen and carbon excretions.

The first serious attempt to determine the influence of pregnancy on the nitrogen balance was that of O. Hagemann (*Arch. f. Anatomie u. Physiol., p. 577 (1890)*) in observations on dogs. When the amount of nitrogen delivered at birth in the newborn, placenta, cord, etc., was considered, it was seen that there was a considerable loss from the mother's body. A. Ver Eecke ten years later (*Mémoires courronés et autres mémoires, Acad. Royale de Méd. de Belgique, Series 4,* **15,** *No. 7 (1900–1901)*) came to the same conclusion from the study of rabbits and announced that "gestation constitutes a sacrifice of the individual for the species."

Paul Bar ("Leçons de pathologie obstétricale," *Vol. 2, p. 271, Paris (1907)*) did not agree with Hagemann and Ver Eecke, but concluded from his own experiments on 5 dogs and several rabbits that "given a sufficient ration, the healthy mother's body can supply the needs of the developing fetus or fetuses without drawing upon its own capital, even when these needs are extreme."

Bar's work was not known to me because he had not published in any obstetrical journal so far as I could find at the New York Medical Library, but had confined his observations to his textbook. However, his work was discovered after my experiments on nitrogen balances in 5 different pregnancies had been completed. Then it was observed that he had neglected the influence of menstruation, and did not consider it important to supply a constant intake of protein and calories for the whole period of pregnancy.

It was the more gratifying to find my results, in the main, in harmony with so careful an observer.

In complete experiments on 2 dogs I found as Bar had done a phase of diminished retention of nitrogen within the first four-week period. Dog A did not show a plus nitrogen balance until after the fourth week and B did not show it until after six weeks. Since the total amount of energy in the food was almost exactly proportional to the animals' weights and the nitrogen intake was not widely different, it is significant that the total amount of nitrogen lost up to the end of the fourth week in the 2 cases is nearly proportional to the weight of the newborn delivered, *i.e.*, 6.0:280; 28:1400.

This itself strongly suggested that the cause of the minus balance was the presence of the embryos. We had as yet no proof of this, however, because we were not certain that the dog would have been in nitrogen equilibrium on the same diet just previous to menstruation and pregnancy.

Dog A therefore was placed on the same measured diet as before, but started some weeks before the expected return of the estrus cycle. Collection and analysis of the excreta began at the same time. First menstrual blood was observed at the end of the fourth week of the diet and copulations occurred one week later. The diet was entirely adequate to maintain the mother in nitrogen equilibrium during sexual rest.

The plus balance began to rise with the first signs of menstruation and continued through the next week. The influence of the menstrual period was, clearly, to cause increased retention of nitrogen. Both Schrader (1894) and Schöndorff (1897) had given as their opinion that retention at this time was a compensation for the loss of blood.

In the final four weeks of pregnancy in the dog it can be shown that on a steady diet the curve of nitrogen elimination in the urine shows that retention of nitrogen is fairly even from hour to hour.

Later I learned Folin's methods for fractionation of the several nitrogenous substances in the urine, and applied them to the urine of pregnancy both in the dog and in several normally pregnant women.

Nitrogen balances in pregnant women had rarely been kept in these early years of the twentieth century. Most of the chemical work on pregnancy urines had to do with

late pregnancy, and both pathologists and obstetricians had formed the belief that normal pregnancy produced a functional derangement from nonpregnancy standards. Almost everyone noted the low percentage of urea nitrogen in the urines and increased percentages of undetermined nitrogen. J. Ewing and C. G. L. Wolf (*Am. J. Obstet.* **55,** *289, see esp. p. 293 (1907)*) and many others were led astray because they had not kept nitrogen balances on their pregnancy cases. If they had done so, it would have been apparent that *the lowest percentage of urea nitrogen occurs simultaneously with greatest nitrogen retention by the product of conception.* It took a novice to furnish the explanation of the so-called "functional derangement."

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GLUCAGON

It is now generally recognized that pancreatic extracts contain a protein factor which is capable of raising blood glucose, presumably by stimulating hepatic glycogenolysis. This factor which C. P. Kimball and J. R. Murlin called "glucagon" (*J. Biol. Chem.* **58,** *337 (1923)*) recently has been separated from insulin by selective precipitation, and isolated in crystalline form (A. Staub, L. Sinn, and O. K. Behrens, *Science* **117,** *628 (1953)*). Whether glucagon has its origin in the alpha cells of the islets of Langerhans, and in cells resembling alpha cells in the gastric and duodenal mucosa, is still a controversial subject (I. J. Pincus and J. Z. Rutman, *Arch. Int. Med.* **92,** *666 (1953)*).

Another important question about glucagon relates to the possible effect of this substance upon the clinical picture of diabetes mellitus. The observation frequently has been made that patients who survive total pancreatectomy need relatively small amounts of insulin (25 to 40 units) for control of their blood sugar level (E. A. Gaston, *New Engl. J. Med.* **238,** *345 (1948)*). However, such patients frequently are insulin-sensitive and, when insulin is withheld, tend to develop ketosis readily. In this latter respect, pancreatectomized individuals resemble young labile diabetic patients. By contrast, certain obese and middle-aged diabetic patients require rather large amounts of insulin to control their hyperglycemia, and yet do not develop ketosis readily when insulin is withdrawn.

When dogs are made diabetic by means of growth hormone (F. G. Young, *New Engl. J. Med.* **221,** *635 (1939)*), or alloxan (E. Thorogood and B. Zimmerman, *Endocrinology* **37,** *191 (1945)*), the insulin requirement is quite high, but resistance to ketosis also is said to be high. When such animals are then depancreatized the insulin requirement decreases; however, the tendency to acidosis increases. These findings and many others have suggested that the clinical and experimental picture of diabetes is not merely due to insulin lack, and that glucagon (and possibly other factors) may play a role in the diabetic state.

Studies of the mechanism of action of glucagon so far have failed to clarify this role appreciably. It appears that the hyperglycemic effect of glucagon is due to hepatic glycogenolysis. E. W. Sutherland and C. F. Cori (*J. Biol. Chem.* **188,** *531 (1951)*) have shown that glucagon increases the activity of hepatic phosphorylase. When glucagon is added to liver slice preparations more rapid liberation of glucose occurs than in a control preparation. However, it has not been possible to demonstrate any effect of glucagon on acidosis (J. Rutman, I. J. Pincus, L. Brown, and M. Scott, *Fed. Proc.* **12,** *121 (1953)*). Under certain circumstances, glu-

cagon may prevent the fall in blood sugar which normally follows administration of a small amount of insulin (Pincus and Rutman, *loc. cit.*)

W. R. Kirtley, S. O. Waife, O. M. Helmer, and F. B. Peck (*Diabetes* **2,** *345 (1953)*) have reported on the administration of glucagon to normal and diabetic human subjects. They used a highly purified preparation extracted by the method of Staub *et al.* (*loc. cit.*), and employed an empiric dose of 20 units per kilogram of body weight. (In terms of biologic activity the unit is that amount of material per kilogram of body weight which, on intravenous administration into a twenty-four-hour fasted anesthetized cat, produces a blood sugar rise of 30 mg. per cent within twenty-five minutes.) These investigators gave the glucagon intravenously over a thirty-minute period to normal subjects, stable diabetic subjects and unstable diabetic subjects. On another occasion, epinephrine was injected into the same subjects in order to compare the metabolic effects of the two substances. Blood glucose, pyruvate, lactate, inorganic phosphate and potassium were determined.

In the normal subjects blood glucose continued to rise until the glucagon infusion was terminated. Glucose values then fell rapidly over a period of thirty to sixty minutes to a point below the initial level. Serum inorganic phosphate levels dropped significantly while blood glucose rose. Pyruvate failed to rise and, indeed, fell after glucagon injection. After epinephrine injection, blood glucose rose and inorganic phosphate fell; however, pyruvate values increased, displaying a pattern which paralleled that of glucose. Stable diabetic subjects responded to glucagon and to epinephrine in a manner roughly similar to that of the normal subjects. By contrast, unstable diabetic subjects failed to show an appreciable rise in blood glucose after glucagon administration, although they demonstrated a striking increase in blood glucose after epinephrine administration. Some increase in steroid excretion took place during the brief period of glucagon injection, but the findings were difficult to evaluate because of the acute conditions of the experiments.

Kirtley and co-workers interpreted the degree of rise they observed in blood glucose after glucagon administration as being related to the amount of liver glycogen immediately available. Hence, the small rise in the unstable diabetic patient was believed to be due to a decreased quantity of liver glycogen in such individuals. Had this been the case, however, epinephrine might also have been expected to elicit a smaller than usual rise in blood glucose in unstable diabetic subjects. Actually, the rise in blood glucose was considerable. Failure of pyruvate levels to increase after glucagon was given indicated that glycogenolysis in muscle did not take place, and that the glucose derived from liver glycogen may have been deposited promptly as muscle glycogen.

Kirtley *et al.* did not report the effect of glucagon on lipid metabolism in man. E. S. Haugaard and W. C. Stadie (*J. Biol. Chem.* **200,** *753 (1953)*) have shown that glucagon decreases the hepatic synthesis of fatty acid from labeled acetate. Pincus and Rutman (*loc. cit.*) have concluded from their own studies on dogs that either glucagon has no effect on lipid metabolism, or that any effect produced by it is an indirect one.

In attempting to summarize what is understood about the physiologic role of glucagon, one finds that there are many unanswered questions. For example, it is not clear whether glucagon acts to inhibit glucose utilization in the periphery. The evidence on this point is conflicting. Glucagon has been shown to increase the width of the epiphyseal cartilage of the hypophysectomized rat (H. Elrick, *Proc. Soc. Exp. Biol. Med.* **82,** *76 (1953)*). Accordingly, the effect of this substance on growth requires further investigation. Although it seems that glucagon is hormonal in nature and plays a role in the normal regulation of blood sugar (J. Bornstein, E. Reid, and F. G.

Young, *Nature* **168,** *903* (*1951*); P. P. Foà, H. R. Weinstein, and J. A. Smith, *Am. J. Physiol.* **157,** *197* (*1949*)), the actual site of origin of this substance and its relationship to a possible trophic factor in the anterior pituitary remain to be defined. Nor is it possible at this time to state what part, if any, glucagon plays in the clinical picture of diabetes, its variants and its complications. The present view seems to be that glucagon is more important as a modifying factor in the patient with diabetes than as a causative agent of diabetes. Studies in progress with the crystalline preparation should add much to our knowledge of glucagon in the coming year.

EFFECTS OF PROTEIN ADDITION TO LOW CALORIC DIETS

An important objective in military nutritional research is the development of a ration which will permit survival on the least possible dietary bulk. In general such rations provide a submaintenance supply of calories. The advantages to be gained by the addition of protein to subcaloric diets and the influence of fluid restriction on the metabolic response to such diets have been carefully studied at the Quartermaster Climatic Research Laboratory (*see Nutrition Reviews* **11,** *327* (*1953*)). The results of balance studies in volunteers ingesting such diets have been reported by M. Quinn, C. R. Kleeman, D. E. Bass, and A. Henschel (*Metabolism* **3,** *49*, *68* (*1953*)).

Two groups of 5 healthy adult males served as subjects for the first study. One group ate a synthetic diet with a daily caloric content of about 900 calories and 43 g. of protein. The second group ate an isocaloric diet with only 0.17 g. of protein. In the low protein diet the calories from carbohydrate, but not from fat, were increased. Fluids were restricted to 800 ml. per day. Complete nitrogen, sodium, chloride, potassium, phosphorus and water balance measurements were made during nine days on the test diets.

The mean weight loss was 5.5 and 4.1 kg. for the protein and nonprotein groups respectively. Both groups were in negative nitrogen balance throughout the period of study. The mean negative nitrogen balance for the group fed protein was 56.4 g. and in the nonprotein group it was 59.9 g. This difference was not considered to be significant.

Differences in water balance of subjects on the two diets were observed. The mean external water balance was determined by subtracting the insensible water loss and fecal water from the water drunk and the water of oxidation from food. This figure was 5703 ml. in the protein-fed group and 4130 ml. in the nonprotein group. The increased urine volume was attributed to the larger amounts of nitrogenous solutes excreted in the urine. An analysis was made of the source of the excess water lost by the protein-fed group which amounted to 1573 ml. Seven hundred and forty ml. could be attributed to the oxidation of larger amounts of fat and about 840 ml. to a greater loss of body water. Additional evidence of increased fat consumption was obtained from skinfold measurements and the presence of ketonuria in the group on the protein diet.

The compartments of body water were measured in the two groups. Similar absolute losses of blood, plasma and extracellular volumes occurred. It was only from the intracellular fluid that the subjects on the protein-containing diet lost more water.

The analysis of the electrolyte data is complicated by the fact that the mineral contents of the two diets were not the same. The authors state that in view of the availability of the various mineral constituents from tissue catabolism the differences in intake of potassium and phosphorus in the diets were of little consequence. The most

interesting finding observed was a shift of potassium and sodium into the intracellular compartment on both diets.

A second study was made using similar diets and a more liberal water intake to determine whether the failure of the protein-containing diet to improve nitrogen balance and maintain fluid balance could be attributed in part to the restricted fluid intake allowed during the first experiment. The size of the groups, the experimental plan and the character of the diet were kept the same except that the subjects were allowed 27 ml. of water per kilogram of lean body mass. The losses of weight and nitrogen were not significantly affected by the increased fluid intake. The mean daily negative nitrogen balance was 6.3 g. and 6.7 g. for the protein- and nonprotein-fed groups respectively. The acute water loss and mild dehydration which occurred early in semistarvation were not corrected by the increased water. The external water loss in the two groups during the entire study period, however, was similar. This is to be compared with the first experiment in which the group receiving the protein-containing diet lost more fluid. There was no significant alteration in the electrolyte patterns between the first and second experiments. Ketonuria again was found in the group receiving protein in the diet.

Quinn and his associates point to the differences between their findings and those of D. Schwimmer and T. H. McGavack (*Quartermaster Food and Container Institute Report No. 5, August 1948*). In the latter experiments using a similar caloric and fluid intake, 800 ml., an increase in protein content from 18.7 to 45 g. daily resulted in a decrease in the negative nitrogen balance. In trying to explain the observed experimental discrepancy Quinn *et al.* discard a number of possibilities such as (1) the quality of the protein fed, (2) the status of the "nitrogen stores" before the study began, (3) improper time relations in the feeding of protein and nonprotein calories, and (4) the mineral content of the diet. None of the above factors would seem to explain the failure of protein in the diet to exert a beneficial effect, and this underlines the importance of total caloric intake as a protein sparer. When a considerable caloric deficit exists, amino acids are used almost exclusively as a source of energy.

The presence of ketonuria in the subjects eating the protein-containing diet is not easily explained in light of the observation of J. L. Gamble ("Chemical Anatomy, Physiology and Pathology of the Extracellular Fluid," *5th edition, Harvard University Press, Cambridge (1947)*), that 50 g. of carbohydrate will almost prevent ketonuria in fasting. Although the protein-containing diet provided 70 g. of carbohydrate as compared to 116 g. for the nonprotein diet, this difference in carbohydrate content of the diet probably is not the sole factor responsible for ketonuria. A direct ketogenic effect of the ingested amino acids would seem possible. An indirect effect of protein on ketogenesis is suggested by the authors whereby increased deamination leads to the formation of increased amounts of glutamic acid through a reaction of the amino groups with alpha-ketoglutarate. The syphoning off of Krebs cycle intermediates, according to this view, would slow the rate of acetate oxidation and lead to the formation of excess ketone bodies.

METABOLISM IN MULTIPLE MYELOMA INFLUENCED BY ACTH

The malignant disease multiple myeloma exhibits, clinically, lesions in the bone, profound alterations in the plasma proteins, usually with the appearance of an abnormal protein, proteinuria, and irregular although often marked changes in nitrogen, calcium and phosphorus balances. The effect of ACTH administration upon this disease has

been studied, but the results have not been entirely consistent. The careful metabolic study of 3 patients with multiple myeloma by W. S. Adams, E. D. Mason, and S. H. Bassett (*J. Clin. Invest.* **33,** *103 (1954)*) is a welcome addition to our knowledge.

Standard chemical and metabolic methods were used, although the plasma proteins were not determined by one of the more modern methods of fractionation but by sodium sulfate precipitation. This is unfortunate because one of the most striking changes to be expected is in the relative proportions of the several plasma protein fractions. Electrophoresis of the plasma proteins might have added to the precision of the results. The authors were aware of this limitation in the method used.

The negative nitrogen, phosphorus and potassium balances which it is customary to see associated with ACTH or cortical steroid therapy were well duplicated in the 3 patients studied. One patient who had the expected profound negative nitrogen, phosphorus and potassium balances associated with ACTH therapy was given testosterone while the pituitary hormone was continued. This addition reduced the urinary nitrogen, phosphorus and potassium excretions so that equilibrium or positive balance ensued. This patient showed the most striking clinical effect, with a complete remission in the manifestations of the disease except for the continued presence of myeloma cells in the bone marrow. He also had a striking reduction in urinary protein excretion (from 8 g. to approximately 1 g. per day). A gradual reduction in the serum protein concentration likewise occurred, chiefly a reflection of a reduction in serum albumin concentration rather than of the other serum proteins.

Clinical improvement did not occur in the other 2 patients. One, in fact, died with infection of unknown cause which began after fifteen days of ACTH treatment. He likewise demonstrated a profound negative nitrogen, phosphorus and to a less extent potassium balance during ACTH therapy and also a reduction in plasma protein, which in this case was due almost entirely to a reduction in the gamma globulin component. There was little or no reduction in the urinary protein excretion.

The third patient showed a marked subjective improvement during ACTH therapy, although the course of the disease did not seem to be altered. Negative nitrogen and phosphorus balances were seen again in this patient. The potassium balance was not studied. He did have a striking reduction in the urinary protein excretion (from approximately 16 to 18 g. to about 4 g. daily). He showed consistent but definite reduction in plasma proteins during the course of therapy which was accounted for chiefly by a fall in the alpha globulin fraction.

The calcium balances are a little difficult to interpret. In the patient who died there was a fairly pronounced negative calcium balance during the last five to ten days of ACTH therapy. The authors suspected that this might be associated with his acute febrile illness. The other patients appeared to have inconsistent and variable changes in calcium balance. Since there were striking changes in the plasma proteins as well as in nitrogen and phosphorus metabolism, the changes in calcium balance observed are difficult to relate to any one phenomenon.

Two of the patients developed a mild psychosis during ACTH administration. There appeared to be no alleviation of this reaction or of the negative nitrogen and phosphorus balances by the administration of relatively large amounts of potassium chloride by mouth during short periods.

It is impossible to make any decisive conclusions from this study. Nevertheless it seems evident that these patients with multiple myeloma responded metabolically to ACTH much as other individuals have. In one instance a remission in the clinical manifestations of the disease occurred during therapy and lasted following its withdrawal. The complication of fatal infection can occur in multiple myeloma as in other conditions

treated with ACTH or with adrenal cortical hormones. Finally, changes were usually seen in the serum proteins and in two instances there was a striking reduction in urinary protein excretion associated with ACTH therapy.

FACTORS INFLUENCING CAROTENE AND PROTEIN CONTENTS OF ALFALFA

An extensive comparison has been completed concerning the influence of variety and certain environmental factors on the carotene and protein contents of alfalfa, a principal hay crop of the irrigated west, and an important hay and forage crop throughout the country. This study was recently published by L. G. Jones, F. P. Zscheile, and R. B. Griffith (*Hilgardia* **22,** *179* (*1953*)). An adequate sampling method was devised for study of the whole culm, including both stem and leaves, and for comparison of varieties according to physiologic maturity rather than with respect to identical ages.

Six standard varieties were studied during six successive cuttings, usually sampled at the one-tenth bloom stage. No variety was consistently higher than others in either carotene or protein content. Other comparisons between 16 varieties and strains, but involving fewer cuttings, confirm this similarity. Any differences found could be attributed to differences in leafiness. A single third-cutting comparison of 23 varieties and 15 selections presented ranges of 190 to 274 parts per million (p.p.m.) carotene and 16.0 to 22.6 per cent protein. In general, protein values were parallel to carotene contents. Selections from India were below average, those from Africa and Argentina were higher than average, while Chilean strains were about average.

Physiologic factors were very important. Whenever a sufficient number of cuttings was sampled, a definite seasonal trend was observed. An upward trend in carotene content, amounting to 65 per cent, was observed during the entire year. The maximum value was obtained in late summer, for the sixth cutting. From June to October the carotene content of alfalfa cut at the one-tenth bloom stage increased 23 per cent. Some variation occurs during the day and from day to day. No similar trends were evident in protein content. This was true for the average of 6 standard varieties. California Common showed a similar increase of 14 per cent in a shorter period of twelve weeks. These data show that considerable error may arise from comparisons among different varieties sampled at different seasons of the year.

The total weight of each culm increases steadily as the cutting develops, until well after seed pods are formed and thus long past the time of cutting for hay. This is largely due to increase of fiber content. The protein and carotene contents thus decrease with maturity, due to the decreasing proportion of leaves, which contain most of both protein and carotene. When total carotene per culm is considered, however, the rise in carotene is progressive well past the normal cutting time of one-tenth bloom and decreases only when seeds begin to ripen and leaves become senescent and shattered.

Since varieties differ widely in maturity dates, the practice of sampling them at uniform times after cutting can lead to very erroneous conclusions, with regard to their carotene-producing capabilities. It appears that in breeding programs designed to obtain improved alfalfa strains, emphasis should be placed on obtaining greater leafiness and that great care must be observed in sampling for comparison purposes.

The influence of moisture content on the amount of leaf shatter losses during alfalfa hay curing was reported by J. B. Dobie,

L. G. Jones, and F. P. Zscheile (*California Agriculture* **7**, *No. 4, p. 4 (1953)*). Raking hay at the wrong time may cause extreme loss of leaves, reducing the yield as much as 20 to 30 per cent. The quality suffers even more because most of the nutrients are found in the leaves. Loss by shattering is most pronounced under conditions of low humidity when the hay is handled at a moisture content below 55 per cent. Maximum yield may be obtained by raking when the hay reaches a dryness of between 55 and 65 per cent moisture. Then maximum carotene and nearly maximum protein retentions will result.

The effect of cutting treatments on the carotene content of irrigated Ladak alfalfa in the Yakima Valley was studied by W. S. McGuire and J. A. Jackobs (*Agronomy J.* **43**, *379 (1951)*). They found yield of dry matter the most important factor associated with carotene content, which decreased at the rate of 43 micrograms per gram for each 1000 pounds gain in dry matter. Highest total seasonal carotene with adequate carotene content for the market was produced when the yield per cutting was ½ to 2 tons per acre. Spring clipping, previous fall cutting and interval between cuttings had little direct effect on carotene content. The interval had an indirect effect only in relation to yield of dry matter. A seasonal variation was observed, with lowest values in late June. In the Yakima Valley it is common practice to cut alfalfa for dehydration in the bud stage because any further increase in dry matter leads to poor quality in the product.

The carotene and nitrogen contents of alfalfa forage from selected clonal lines have been compared by E. V. Staker and B. H. Crandall (*Agronomy J.* **45**, *66 (1953)*). They compared the variety Grimm to 11 clones, some having Turkestan germ plasm and others that trace back to variegated varieties. Only two sampling periods were employed, the prebud stage and the early-bloom stage. Results failed to show significant differences in carotene content of clonal lines selected for factors other than carotene. Nevertheless these workers express the opinion that a breeding program designed primarily to develop lines high in carotene would prove successful and that carotene and nitrogen contents are interdependent.

All of these results are in essential agreement so far as they can be compared. An understanding of the factors considered should lead to practical benefits in producing improved products from alfalfa, an intrinsically very valuable food plant for livestock. Much can be gained by the proper handling of such a crop, or it may be greatly deteriorated through neglect of proper precautions. These procedures are then reflected in the nutritive value of the product.

VITAMIN CHANGES IN DAIRY PRODUCTS AND PLANTS ON GAMMA IRRADIATION

A possible new use for products of large-scale atomic enterprises is food sterilization by gamma-radiation. The stability of various vitamins during irradiation by gamma-rays from Co^{60} was reported by H.-C. Kung, E. L. Gaden, and C. G. King (*J. Agr. Food Chem.* **1**, *142 (1953)*). They irradiated fresh and evaporated milk, cream, cheese, butter, and margarine for periods of one to twelve hours at dosages of 80,000 to 2,000,000 roentgens.

Vitamins A and E, carotenoids, ascorbic acid and riboflavin were considerably destroyed, but the enzyme phosphatase was inactivated only slightly. In raw milk, carotenoids, riboflavin, tocopherols, vitamin A, and ascorbic acid were destroyed 5, 10, 30, 30, and 78 per cent, respectively. De-

struction increased rapidly with time, reaching 45 per cent with carotene and riboflavin, and 85 per cent with vitamin A in twelve hours. In only six hours, tocopherol had been 60 per cent destroyed and ascorbic acid 100 per cent. Rates of destruction were somewhat less in evaporated milk for ascorbic acid and vitamin A but comparable for carotenoids and riboflavin. Linear relationships between the logarithm of concentrations of vitamins and irradiation time indicate first-order reactions under the conditions studied.

Destruction of vitamin A occurred faster in the following order of mediums: margarine, cream, cheddar cheese, cream cheese and butter. The destruction was three-fold greater in butter than in margarine for all time periods studied, a very significant difference, since 80 per cent of the vitamin A content of butter was lost in twelve hours.

The total dosage was within the approximate range required for sterilization of mixed bacterial cultures. Such generalized destruction of vitamins would seem to limit the commercial possibilities for such a process unless dosage could be reduced and still effect sterilization.

In contrast to the rapid destruction of ascorbic acid in dairy products, A. R. Cooke (*Science* **117,** *588* (*1953*)) has reported a striking increase of the ascorbic acid content of green plants when irradiated with gamma-rays from Co^{60} (300 curies at a distance of 1.3 meters). He exposed snapdragon plants continuously for periods up to ten days to an intensity of 5000 roentgens per day. Progressive increases of ascorbic acid content were observed up to 70 per cent over the controls (nonexposed plants) during a nine-day period. No visible damage to the plants was observed up to this time, though it was apparent later. Soybean plants were exposed to x-rays at 1000 roentgens per minute. In this way acute doses of 1000, 4000, and 16,000 roentgens were applied. For all doses, a small (4 to 10 per cent) decrease occurred two days after exposure, but then the content began to rise, to show a gain of 34 per cent in five days with the greatest dose. Gains were proportional to the dosage in this range.

Other work has shown that ascorbic acid may protect animals from injury due to such radiation, possibly because certain enzyme systems were protected. Cooke noticed that there was a positive correlation between the initial ascorbic acid content of plants and their relative resistance to irradiation. Eight species of plants were grouped in such a correlation. Cabbage was one of the most resistant and it is well known as a good source of ascorbic acid. This resistance does not depend upon the increase of ascorbic acid content during exposure but only on the initial content. Such work should be extended to other plants. An understanding of this protection may lead to methods of protecting life against very large doses of high-energy radiation.

PROTEIN CONSTITUENTS OF CORN

Since corn grain protein is deficient in tryptophan, lysine and niacin, the possibilities of improvement in these respects were investigated from the nutritional viewpoint of the stock feeder, as well as of those interested in human nutrition, by P. A. Miller, T. L. Hurst, and B. Brimhall (*Agronomy J.* **44,** *343* (*1952*)). Illinois high-protein and low-protein corn strains, their crosses and backcrosses were compared as to contents of total protein, zein, tryptophan, lysine, leucine, valine, isoleucine and niacin. It appears that lysine and the other protein components are not directly related and that lysine determination is the only basis for selection of corn strains with higher lysine percentage.

Zein contains little lysine or tryptophan;

therefore it would be desirable to increase the corn content of these two amino acids without increasing the amount of zein. A good opportunity for such genetic selection was found in the material studied. Selection for increased crude protein content may merely increase zein. Thus it appears more desirable to select directly for higher contents of lysine and tryptophan.

When niacin is added to corn rations, the utilization of tryptophan by the animal body may be greatly increased. Selection for this constituent would thus be desirable also. In this study, niacin content also was found to be essentially independent of protein or its components. Corn protein quality thus appears capable of improvement by plant breeding methods.

Other comparative studies were made on the contents of tryptophan, niacin, indoleacetic acid, reducing sugars, soluble polysaccharides and sucrose in two series of corn kernels carrying starchy and sugary genes. H. J. Teas, J. W. Cameron, and A. C. Newton (*Agronomy J.* **44,** *434* (*1952*)) reported these contents during the period of kernel development. One series provided whole starchy kernels from the inbred line, and sugary kernels from a backcross product having fifteen-sixteenths inbred germ plasm. In the other series both sugary and starchy kernels were taken from the same ear at each given age. This stock carried a reciprocal translocation involving marker genes; only endosperm tissue was used in this series.

In both series, the substances studied were higher on a per kernel basis in sugary than in starchy kernels during the later part of the developmental period; at early stages all were equal in the two genotypes. At maturity niacin, reducing sugars, sucrose and water-soluble polysaccharides remained higher in sugary kernels, tryptophan was slightly higher and indoleacetic acid showed no difference. At thirty days after pollination, tryptophan, indoleacetic acid and niacin reached maximum values. Reducing sugars and soluble polysaccharides were high at this period. It was not possible from these ontogenetic data to conclude whether tryptophan functions as a precursor of niacin and indoleacetic acid in the corn kernel, or whether these substances are themselves translocated into the kernel.

DIETARY PROTEIN AND FAT CONTENT OF FECES

Absorption of ingested fat is dependent, in part, upon the quality and quantity of bile excreted into the intestinal tract. Since certain proteins and amino acids may increase the output of cholate in bile, D. F. Magee, K. S. Kim, and A. C. Ivy (*Am. J. Physiol.* **175,** *310* (*1953*)) have investigated the effect of ingested casein on the fecal excretion of fat.

In one series of experiments 15 dogs were divided into three groups of 5 animals each. One of three basal diets was fed to each group. One diet consisted of corn oil, corn starch, sucrose, cellu flour and salt mixture (Wesson) in proportions such that fat made up 27 per cent, by weight, of this diet, while the protein content was low. Another of the basal diets was fat-free and consisted of vitamin-free casein, cellu flour, corn syrup solids and the salt mixture. In the third diet, part of the corn syrup solids was replaced by corn oil, so that 9 per cent of this diet, by weight, was fat. Each dog received an amount of food equal to 80 calories per kilogram per day and a multivitamin capsule every two days. After the dogs were on the basal diets for one month or longer, during which time they remained in apparent good health, the study to evaluate the effect of ingested casein on fecal fat excretion was begun. An eight-day metabolic period during which the basal

diets were fed was followed, or preceded, by a period of similar length during which casein was substituted in the diet for an equal weight of carbohydrate. The substitution amounted to 12 per cent (by weight) of the diet in the low protein, high fat diet and 20 per cent in the other two diets. The feces were collected and analyzed for the last six days of each feeding period.

Somewhat less fecal fat was excreted by each of the three groups of dogs during the extra-dietary casein periods. The P values for the differences of the means of the casein-supplemented and nonsupplemented periods (calculated from the distribution of Fisher t) were less than 0.05 in each instance. The differences in fecal fat excretion were slight in terms of fat ingested. The data indicate that the so-called endogenous fat may not be a fixed quantity, but may vary, as does the so-called exogenous fecal fat, with alterations in the dietary casein level. However, the differences in fecal fat excretion were slight in terms of fat ingested among the dogs fed the diets containing 9 per cent or 27 per cent fat.

Greater differences were observed in a second series of experiments carried out in rats. Two groups of rats received basal diets consisting of 6 per cent corn oil by weight, 16 per cent casein, 17 per cent sucrose, 3.5 per cent cellu flour, 3.5 per cent salt mixture and a vitamin supplement. In addition, one group of 10 rats received extra sucrose in an amount such that the sugar ingested amounted to 54 per cent of the total diet; the other group of 10 rats received, instead of the extra sucrose, casein in an equal quantity. Eight and one-half g. of each diet were given daily to each rat. Each group ingested all its respective diet for twenty-five days, during the last twenty-one of which the feces were collected. The fecal fat was, on the average, significantly less (P value less than 0.01) in the group ingesting the high protein diet. During the twenty-one days of observation, an amount of fecal fat equivalent quantitatively to about 15 per cent of the ingested fat appeared in the feces of the rats on the low protein diet, while about 6 per cent appeared in the feces of the rats on the high protein diet.

Magee and his co-workers are of the opinion that significant differences in intestinal mobility probably did not occur during the different dietary regimens, since the weight and consistency of the feces were the same among the various comparable groups. They suggest that the lessened fecal fat excretion observed in the dogs when additional casein was fed may have been due to a greater absorption of fat secondary to the action of casein in increasing the daily output of cholate in the bile in this species (D. F. Magee, K. S. Kim, and A. C. Ivy, *Am. J. Physiol.* **169,** *317 (1952)*). However, the quantity of sucrose in the diet, as well as its protein content, must be considered since F. F. Coburn and J. Annegers (*Ibid.* **163,** *48 (1950)*) have found in dogs that while sucrose does not appear to alter endogenous or fasting cholate synthesis, sucrose inhibits the action of exogenous cholepoietic substances, including casein. In the diets used by Magee and co-workers, when protein was high, sucrose was low; when protein was low, sucrose was high. Another possible mechanism considered by Magee *et al.* for the lessened fecal fat excretion during the periods of increased casein ingestion is suggested by the work of Grossman *et al.*, who observed an increase in pancreatic lipase excretion during casein administration to rats.

Ingestion of protein may not always lead to a decreased fecal fat excretion. R. A. McCance and C. M. Walsham (*Brit. J. Nutrition* **2,** *26 (1948)*; *Nutrition Reviews* **7,** *88 (1949)*) studied the apparent digestibility of fat in whole wheat. They found a mean digestibility of fat of about 60 per cent that for wheat. It was suggested by them that this low digestibility of wheat fat may be due to the difficulty with which lipolytic

enzymes penetrate the vegetable cells. However, in patients with celiac disease, ingested wheat gluten also inhibits the absorption of added oils consisting of either saturated or unsaturated fatty acids (*Ibid.* **11,** *199* (*1953*)). In this circumstance, the factor of penetrability of the vegetable cells is not involved. The adverse effects on fat absorption of certain dextrins and starches in patients with celiac disease is also well known (*Ibid.* **9,** *324* (*1951*)).

The observations of Magee, Kim, and Ivy on the effect of dietary protein on fecal fat excretion point out again the mutual interdependence of various food components. Further studies are needed to determine the optimal dietary protein:fat:carbohydrate ratios, not only for the normal subject, but, more particularly, for patients with specific or nonspecific (*e.g.*, following serious injury) nutritional problems.

DIETARY INTAKE AND ALCOHOLISM

In a previous discussion (*Nutrition Reviews* **11,** *212* (*1953*)) it was indicated that certain inadequacies may have existed in the original experiments demonstrating the "genetotrophic" concept of alcoholism. Another attempt to explore the relationship between dietary intake and alcohol ingestion is that of W. W. Westerfeld and J. Lawrow (*Quart. J. Studies Alcohol* **14,** *378* (*1953*)), who investigated the effect of restricting the intake of food and of reducing the thiamine intake upon the desire for alcohol.

Alcohol intake was studied in weanling female rats which were given an ad libitum diet and subsequently were given only 50 or 75 per cent of the food eaten in the ad libitum period. Thus, one group was allowed the ad libitum intake and the second and third groups of 6 rats each were allowed only 75 and 50 per cent of their ad libitum intake respectively. Rats of the first and second groups did not increase their alcohol consumption significantly, but the 50 per cent group did so markedly. Five of the 6 rats in this category ingested more alcohol, resulting in an average ingestion of 22 ml. daily as compared to the 4 ml. which they drank during the control period. When these animals were allowed 100 per cent of their ad libitum intake a prompt decrease in alcohol consumption followed. Similarly, when the full ad libitum diet group was restricted to only 50 per cent of ad libitum intake a gradual increase in alcohol consumption occurred. The relationship between reduction in calories eaten and increase in alcohol ingestion was thus apparent only in the animals which had a 50 per cent change in caloric intake. In these animals, however, the ingestion of alcohol calories as compensation for the reduced food intake was sufficient to replace only approximately 40 per cent of the calories lost in the restriction.

In a separate series of studies designed to determine the effect of induced thiamine deficiency and the resultant reduction in food intake upon the desire for alcohol, male rats were divided into three groups of 8 animals each. The control group received a complete synthetic diet, a second group was given the same diet minus thiamine and a third group consisted of animals pair-fed with the thiamine-deficient animals but receiving added thiamine.

During the seven-week duration of this study 2 of the control rats increased their alcohol intakes which, therefore, raised the average alcohol intake of the group. Only 3 of the animals on the thiamine-deficient diet, however, demonstrated a clear increase in alcohol consumption and these 3 survived the longest. The other rats which did not consume alcohol died of thiamine deficiency prior to the end of the study. In the pair-fed group 6 animals showed a marked increase in their alcohol intake which was even

greater than that which occurred in the thiamine-deficient rats. Two of the pair-fed animals did not increase their alcohol intake and died during the study. There was a progressive decrease in the food intake of the thiamine-deficient animals and thus also a progressive decrease in the food intake of the animals pair-fed with these. This reduction in food eaten was not associated with an increase in alcohol ingestion until the caloric intake fell to approximately 50 per cent of the initial level. The control animals showed no change in the amount of water ingested, but the thiamine-deficient animals which consumed alcohol markedly decreased their water consumption from 20 cc. to 2 cc. per day. In the thiamine-deficient rats which did not consume alcohol daily water consumption was 4 cc. and in the pair-fed animals, it was 6 cc. Total fluid intake of water and alcohol in the latter group increased to approximately 34 cc. per day. The results of the thiamine deficiency experiment were unexpected and, therefore, it was repeated in essentially the same manner and 5 more rats were added to the thiamine-deficient group. This repetition revealed similar results so that of a total of 21 thiamine-deficient rats in both experiments only 4 showed an increase in alcohol consumption and these survived longest. In the pair-fed rats, however, 13 of 16 animals markedly increased their alcohol intake.

Caloric intake in the various groups showed marked differences, with 66 calories being eaten by the control animals whereas the thiamine-deficient animals and their pair-fed mates ate progressively less until their daily intake was only 21 calories at the end of the experiment. Alcohol consumption replaced only 15 calories for the pair-fed animals. Thus, only one third of the calories which were withheld were compensated for by the ingestion of alcohol.

This study reveals somewhat unexpected data in view of the fact that W. W. Westerfeld and E. A. Doisy (*J. Nutrition* **30,** *127 (1945)*) had previously observed that alcohol ingestion prolonged the survival of thiamine-deficient rats. In these latter experiments, alcohol drinking was increased in only 2 of the animals with thiamine deficiency. Thus, apparently the combination of reduced caloric intake and thiamine deficiency does not increase the intake of alcohol, although reduction in calories alone clearly increases it. Accordingly, this finding is in conflict with the suggestion of J. Mardones (*Quart. J. Studies Alcohol* **12,** *563 (1951)*) that thiamine, in association with Factor N, is most effective in reducing or preventing increased alcohol intake.

VITAMIN D BALANCE IN THE RAT

Since no satisfactory chemical or physical method is available for the estimation of vitamin D in tissues, the experimenter is forced to employ the laborious biologic assay technics for the determination of vitamin D in tissues. Consequently, there is little information of a quantitative sort as to the sites of storage of this vitamin. E. M. Cruickshank and E. Kodicek (*Biochem. J.* **54,** *337 (1953)*) have undertaken the heroic task of tracing the fate of a massive dose of vitamin D administered to rachitic rats. Piebald rats were depleted of vitamin D by maintenance from weaning on the Steenbock rachitogenic diet. Within three weeks florid rickets had developed. At this time four groups of 5 rats each received a single dose of 40,000 I.U. of ergocalciferol. The fifth group served as deficient controls. One group of treated rats was killed on each of four days following administration. The deficient controls were killed on the fourth day. Urine and feces were collected for assay; liver and intestines were analyzed separately from the remainder of the carcass.

X-ray examination of the tibias of treated

animals showed no signs of calcification of the rachitic bone on the first day following treatment. On the second day some slight trace of healing was visible and by the fourth day it was appreciable. The deficient control animals were shown on assay to contain 12 I.U. of vitamin D, of which 1 I.U. was in the liver, 1 in the intestines and 10 in the remainder of the carcass. Five units were excreted in the feces during one day. Animals killed one day after dosage with 40,000 I.U. contained less than 2400 units of the vitamin. Of this amount more than half was located in the liver. Four days after dosage the vitamin D content of the rats had dropped to less than 1000 units, of which 400 were stored in the liver. Fecal excretion on the first day after treatment amounted to nearly 5000 units. This diminished sharply to less than 1400 units on the second day, 200 units on the third and 30 units on the fourth. No vitamin D was found in the urine. Thus, of the 40,000 units administered, only 7000 could be accounted for on the first day following dosage. Of this, one-third was found in the body and two-thirds in the feces. Since vitamin D losses through scattering of food or through destruction during the extraction process were carefully excluded, it must be concluded that between 70 and 80 per cent of the administered vitamin had been converted into a substance biologically inactive for the rat.

BACTERIAL DEAMIDATION OF NIACINAMIDE

The conversion of niacin to its metabolically active derivatives, diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN), has been studied in mammals and in other organisms. Particularly well adapted for this type of investigation is the bacterium, *Lactobacillus arabinosus*. The DPN content of this organism may be measured by determination of the glycolytic activity of washed cells. The organism does not destroy the coenzyme rapidly. D. E. Hughes and D. H. Williamson (*Biochem. J.* **51,** *330 (1952)*; *Nutrition Reviews* **10,** *312 (1952)*) utilized *L. arabinosus* in studying the bacterial synthesis of DPN and demonstrated that cells may utilize niacin, niacinamide, niacinamide riboside, or niacinamide ribotide equally well as precursors. It was observed in these investigations that when niacinamide was added to the glycolyzing bacterial cells, it disappeared from the medium rapidly and could not be entirely accounted for in the DPN synthesized. There was evidence that niacinamide was being removed by a process of deamidation, since both niacin and ammonia were recovered from the medium. This reaction was found to proceed at a rate approximately two hundred times as fast as the synthesis of DPN.

The deamidation of niacinamide by *L. arabinosus* has been investigated further and the properties of the enzyme responsible for this reaction have been studied in cell-free preparations (D. E. Hughes and D. H. Williamson, *Biochem. J.* **55,** *851 (1953)*). Enzyme activity was determined by measuring the ammonia and niacin released after incubation of washed cells or cell extracts in a medium containing niacinamide and phosphate buffer. The reaction was stopped by addition of suitable quantities of a solution of sulfuric acid, the ammonia was distilled off and measured by a process of nesslerization and niacin was determined in the presence of niacinamide by spectrophotometric measurement of the colored reaction product which results from treatment with cyanogen bromide and sodium hydroxide.

Under standard conditions of culture and determination of enzyme activity, it was found that all strains of lactobacilli studied possessed niacinamide deamidase activity,

but the rate of reaction was greatest with *L. arabinosus* and *L. bulgaricus*, these organisms forming ammonia from niacinamide at rates of from 1 to 3 micromoles per milligram dry weight of bacteria per hour. Uniformly there was a stoicheiometric relationship between the quantities of ammonia and of niacin released. The reaction, therefore, was demonstrated to be a hydrolytic degradation with formation of equimolecular quantities of ammonia and niacin. Under the conditions of testing there was complete splitting of the niacinamide in the medium. The reaction was not inhibited in the presence of high concentrations of either nicotinic acid or ammonia, nor was niacinamide formed when enzyme preparations were incubated with ammonium nicotinate.

Niacinamide deamidase was shown to be a constitutive enzyme of lactobacilli rather than an adaptive enzyme since the ability of cells to hydrolyze niacinamide was independent of the concentration of niacin or its derivatives present in the medium in which the organisms were cultured. The rate of reaction was not influenced by the age of the cells or the presence or absence of glucose in the mediums.

Other bacterial species were tested for niacinamide deamidase activity. Included were strains of staphylococci, streptococci, *Clostridium welchii*, *Escherichia coli*, acetobacter and pseudomonas. With the exception of a single strain of *Staphylococcus albus*, none of these organisms possessed significant enzyme activity.

The properties of the enzyme were studied. It was found that the rate and quality of the reaction were identical in preparations of intact cells and of cell-free extracts. The pH optimum was broad, with consistently high activity between pH 4.5 and 8.0. The substrate specificity of this reaction was amply demonstrated, since no ammonia was released when enzyme preparations were incubated with glutamine, asparagine, arginine, adenine, urea, nicotinuric acid, niacinamide methochloride, trigonelline, niacinamide riboside, DPN, pyridine-3-sulfonamide, and 5-fluoroniacinamide. Because of this high degree of substrate specificity, it was suggested that the enzyme might be utilized for determining the free niacinamide of tissue extracts.

The activity of the enzyme was demonstrated to be much more stable on storage at refrigerator temperatures when present in cell-free preparations than in intact cells. Heat destroyed the activity of all enzyme preparations as did treatment with alcohol, ammonium sulfide and ammonium sulfate. Dialysis did not result in loss of activity.

It was determined that the deamidase reaction of intact cells and of cell extracts was inhibited in the presence of metallic salts, such as copper sulfate, mercuric chloride, and silver nitrate. Although a large number of other compounds were tested for inhibitory properties, none was found. Included among these substances were compounds structurally related to niacinamide and compounds demonstrated to inhibit other enzymatic reactions.

The demonstration of this enzyme in *Lactobacillus* is of interest. Its significance in the economy of the organism or more particularly in the metabolism of niacin is not known. Hughes and Williamson (*loc. cit.*) suggest that niacinamide deamidase is concerned with the regulation of the DPN content of the cells. How this is accomplished is not clear, however, since the same investigators have demonstrated that *L. arabinosus* utilizes niacinamide and niacin equally well in the synthesis of DPN. On the other hand, it is pointed out that certain of the bacterial species which were found not to possess niacinamide deamidase activity are able to split DPN with formation of niacinamide as an end product and perhaps by this means regulate the DPN content of the cells. In contrast, *L. arabinosus* does not contain the enzyme which degrades DPN.

Although niacinamide deamidase seems to

be limited in its occurrence in bacterial species and is found most importantly in lactobacilli, it may well be of significance in animals. Other investigators have claimed that niacin may be derived from niacinamide in the intact animal. It is conceivable that this may result at least in part from the action of lactobacilli in the intestinal tract. However, P. Ellinger and M. M. Abdel Kader (*Biochem. J.* **44,** *627* (*1949*)) and B. C. Johnson and P.-H. Lin (*Fed. Proc.* **10,** *203* (*1951*)) have observed that a significant percentage of parenterally administered niacinamide is excreted in the urine of rabbits and rats. It therefore remains entirely possible that animal tissues may possess an enzyme with activity like that of the bacterial niacinamide deamidase.

COENZYME A AND THE BIOSYNTHESIS OF CHOLESTEROL

It has been established by the use of isotopes that acetate serves as a primary precursor in the biosynthesis of cholesterol in mammals (K. Bloch and D. Rittenberg, *J. Biol. Chem.* **145,** *625* (*1942*)). This process has been studied *in vitro* by the use of liver slices and it has been found that both acetate carbons are utilized and are incorporated in the ring structure as well as in the side chain of the cholesterol molecule (Bloch, *Recent Progress in Hormone Research* **6,** *111* (*1950*)). There is information, also, that acetoacetate may be utilized in this synthesis without prior degradation to acetate (R. O. Brady and S. Gurin, *J. Biol. Chem.* **189,** *371* (*1951*)).

These observations would indicate that the biologic availability of acetate is important to the *in vivo* synthesis of cholesterol. Other types of acetylation are known to be catalyzed by enzyme systems which require coenzyme A, the structure of which includes pantothenic acid (*Nutrition Reviews* **7,** *348* (*1949*)); **9,** *78* (*1951*)). Pantothenic acid deficiency is associated with a decrease in tissue content of coenzyme A and reduced capacity for acetylation (R. E. Olson and F. J. Stare, *J. Biol. Chem.* **190,** *149* (*1951*)). If coenzyme A is important in the control of processes of acetylation which are integral to the biologic synthesis of cholesterol, it would be anticipated that biosynthesis of the sterol would be inhibited in pantothenic acid-deficient animals. K. Guggenheim and Olson (*J. Nutrition* **48,** *345* (*1952*); *Nutrition Reviews* **11,** *216* (*1953*)) sought evidence of such a metabolic disturbance. However, it was found that the cholesterol contents of liver, heart, adrenals and blood serum were similar in deficient rats and in pair-fed control animals. There was no indication, either, that there was a decreased rate of incorporation of carboxyl-labeled acetate in the tissue cholesterol of the deficient animals.

A recent report describes experiments which have approached the problem in a somewhat different way and offers evidence that maintenance of the concentration of cholesterol esters in plasma and in liver is dependent upon an adequate dietary intake of pantothenic acid. These observations are reported by G. S. Boyd of the University of Edinburgh (*Biochem. J.* **55,** *892* (*1953*)).

The animals used were young Wistar rats of both sexes. They were fed a basal diet which was sterol- and fat-free, except for the administration of 0.2 ml. of cod liver oil per animal per week. Water-soluble essential nutrients were contained in adequate quantities in the diet fed. The fat-deficient but otherwise adequate basal diet was modified in three other experimental groups of animals. In the first of these, pantothenic acid was removed from the diet to afford a fat- and pantothenic acid-deficient diet. In another olive oil was added to form 10.5 per cent of the diet, replacing an equicaloric quantity of carbohydrate and providing a diet with adequate fat and pantothenic acid

content. The third modification was one with a like quantity of fat but deficient in pantothenic acid. Animals were fed twice a day and caloric intakes were adjusted to the same level in all groups. Rats on the pantothenic acid-deficient regimen developed manifestations of the deficiency state with growth failure, thinning of the fur and staining of whiskers.

After forty days on the experimental diets the animals were killed and the concentrations of total and free cholesterol in blood serum and in liver were determined. In addition the quantity of coenzyme A in liver specimens was assayed by the procedure of R. E. Handschumacher, G. C. Mueller, and F. M. Strong (*J. Biol. Chem.* **189,** *335 (1951)*).

The coenzyme A activity of liver from rats on pantothenic acid-deficient diets was reduced to approximately half that of animals in the groups fed adequate quantities of the vitamin. Comparative mean values were 109 and 123 units of coenzyme A activity per gram wet weight of liver in the two groups fed diets containing adequate pantothenic acid and 49 and 41 units per gram wet weight in two groups on deficient intakes. The presence of fat in the diet seemed to have no significant influence on coenzyme A levels either with or without the inclusion of pantothenic acid in the food.

The total cholesterol in the plasma was not significantly changed by inclusion of 10 per cent fat in the diet. However, it was reduced by one-third in the animals on the fat- and pantothenic acid-deficient regimen. All of the decrease in total plasma cholesterol was accounted for by a reduction in the ester fraction.

The total cholesterol of liver was increased in the two groups of animals fed the supplement of fat. However, it was reduced by about 25 per cent when pantothenic acid was omitted from the fat-deficient basal diet. Again the reduction was chiefly in the cholesterol esters so that the ester fraction totally disappeared from the livers of some animals and composed only 7 per cent of the mean total cholesterol in the livers of this group. This compared with an ester fraction of from 25 to 45 per cent in other groups. Although the total and ester cholesterol of the livers were lower in the animals depleted of pantothenic acid but given the added fat than in the group given adequate pantothenic acid and fat, it was not ascertained that these differences were significant. The author, therefore, claims that when fat is fed, there is no apparent effect of pantothenic acid deficiency on liver or plasma cholesterol.

Since these animals were placed on sterol-free diets at an early age, it may be presumed that practically all of the carcass cholesterol must have been derived through systems of biosynthesis. The acetate precursor in this instance must have come from the metabolism of glucose. Under the conditions of fat and pantothenic acid depletion in these experiments there was a decrease in the cholesterol ester content of plasma and liver which was associated with manifestations of pantothenic acid deficiency and a reduction in the coenzyme A activity of liver. It would appear, therefore, that coenzyme A through its control of acetylation is directly involved in or is important to the biologic synthesis of cholesterol ester.

The effect of pantothenic acid deficiency on cholesterol ester synthesis was masked or annulled by the feeding of fat in these experiments, a finding which probably also explains the failure of Guggenheim and Olson (*loc. cit.*) to adduce evidence of an effect of pantothenic acid deficiency on cholesterol synthesis.

The author offers the explanation that coenzyme A may be involved in cholesterol synthesis through successive utilization of acetate to form intermediates containing even numbers of carbon atoms. The role of coenzyme A would be reduced in significance if similar intermediates were made available through the catabolism of fat.

This concept is dependent on the consideration that fatty acid derivatives with even numbers of carbon atoms may enter pathways of biosynthesis of cholesterol without being previously broken down to acetate fragments. Evidence has been presented that the four-carbon compound, acetoacetate, may be so utilized.

The experiments reported and the considerations offered are of great interest. It is to be anticipated that carbon-labeled acetate will be utilized to determine more effectively the rates of cholesterol synthesis under conditions of fat and pantothenic acid depletion.

GASTRIC MUCIN AND RAT GROWTH

The gross chemical differences between human and cow's milk have long been well established. That there are in addition more subtle differences has been suspected and concrete evidence is slowly being accumulated. R. M. Tomarelli, E. Linden, G. T. Durbin, and F. W. Bernhart (*J. Nutrition* **51,** *251 (1953)*) have demonstrated that human milk contains a growth factor required by certain strains of *Lactobacillus bifidus* (*see also* P. György, *Pediatrics* **11,** *98 (1953)*; *Nutrition Reviews* **12,** *24 (1954)*), and that cow's milk is a relatively poor source of this substance. The finding that hog gastric mucin contains this same growth factor has made possible studies on the role of this agent in the nutrition of nonruminants.

Young rats (presumably weanlings) served as experimental animals and received a commercial infant formula in liquid form (12 per cent protein, 56 per cent lactose and 28 per cent fat). This preparation contained skim cow's milk with added lactose, fat, vitamins and minerals. The weight gained during eleven weeks on this ration averaged 97 g. The inclusion in the ration of hog gastric mucin at a 5 per cent level increased the weight gain to 145 g. without altering the total food intake over the eleven-week experimental period. This amount of gastric mucin boosted the microbiologic growth factor activity to the level found in human milk. Since the observed stimulation of rat growth by gastric mucin might have been due to increased protein content of the diet, a group of rats was maintained for eleven weeks on the basic infant formula supplemented with acid-hydrolyzed mucin. Acid hydrolysis had been shown to destroy the growth-promoting activity of mucin for *L. bifidus*. The hydrolyzed mucin, while adding significantly to the amino acid content of the ration, did not stimulate rat growth. Similarly, the addition of casein to the basal diet had no significant effect. When a large supplement of B-vitamins was added to the basal infant formula diet, supplementation with mucin still increased the growth performance of the rats. In this case it also increased food intake. Hydrolyzed mucin was again inactive. The growth-promoting action of mucin was also demonstrated with a semisynthetic diet containing 12 per cent casein, 25 per cent fat, 4 per cent salts and 54 per cent lactose.

In an effort to increase the requirement of the experimental animals for the mucin factor, the authors incorporated iodinated casein (Protamone) into the rations. This resulted in drastically reduced weight gains and greatly increased mortality. The addition of mucin again stimulated growth and reduced the mortality rate. The addition of substantial quantities of B-vitamins to the diet containing iodinated casein also reduced mortality and enhanced growth performance. This is not unexpected since thyrotoxicosis is well known to increase the requirements for vitamins of the B-complex. Yet the addition of mucin to the supple-

mented ration still enhanced its growth-promoting activity for rats. Under these conditions, however, it did not influence the mortality rate. The addition of insoluble liver residue to this vitamin-supplemented ration enhanced growth to the same degree as did gastric mucin.

The authors conclude that the growth-promoting effect of added mucin is not due to its protein content but may be due to the factor responsible for the growth of *L. bifidus*. They suggest that the action of the mucin factor may be on the intestinal flora of the rat. The fact that the mucin effect could be demonstrated with a diet excessively supplemented with B-vitamins would seem to rule out the possibility that mucin prevents a bacterially-induced partial deficiency in one of these factors.

COMPOSITION AND DIETARY EFFECTS OF MILKS

Few foods have been the subject of such extensive investigation as milk. Much is known about the composition of human and cow's milk since such information is of immediate practical importance. Relatively little has been done with the milk of common laboratory animals. A broad understanding of lactation will come, however, only after the composition of milk of a wide variety of mammalian species is known.

Milk of ruminants and of many other land animals is characterized by the presence of the low molecular weight fatty acids which are not ordinarily found in depot fats. On the other hand, the fatty acids of the milk of marine mammals are similar to those of blubber fat. J. S. Cama and M. L. Meara (*Biochem. J.* **55,** *365 (1953)*) have determined the component fatty acids of finner whale milk. The milk samples were obtained six to nineteen hours after the whale's death. Four samples were available for analysis. All were viscous and pale pink in color. Analysis by the usual methods showed the fat content to vary between 23 and 55 per cent, water content to vary between 36 and 57 per cent, and nonfat solids to vary between 5 and 20 per cent. The saponification equivalents of the 4 samples were very similar, ranging from 293.5 to 297.3. Iodine values varied between 149 and 177, the lowest value being obtained with the purest sample. The lower molecular weight steam-volatile fatty acids were absent. Lauric acid was present in small amounts in only 1 of the 4 samples. The bulk of the fatty acids was made up of palmitic acid and the unsaturated C_{18}, C_{20}, and C_{22} acids. Thus the fatty acid make-up of finner whale milk does not differ radically from that reported earlier for blue whale milk. There is a reasonably close resemblance between these analytic data for whale milk fats and the data available for whale blubber. Perhaps the most striking difference lies in the higher degree of saturation of the blubber fat. A similar close relationship between milk and blubber fat has been shown in the case of the gray seal.

The biosynthesis of milk fat from acetate and glucose in mammary tissue of rabbits has been studied by G. Popják, G. D. Hunter, and T. H. French (*Biochem. J.* **54,** *238 (1953)*), in order to decide whether mammary glands synthesize fatty acid from nonfat precursors or whether they are synthesized in the liver and then transported to the mammary glands. Both tissues were analyzed for fatty acids and for cholesterol six hours after the administration of C^{14}-acetate and C^{14}-glucose. Since the highest radioactivity in plasma lipids occurred twenty-four hours after the injection of labeled substances, it was argued that prior to the attainment of this maximum, the mammary tissue would show higher specific activity than liver only if the mammary glands were syn-

thesizing fatty acids rather than receiving them from the liver. The investigators were successful in showing that six hours after the administration of labeled acetate or glucose the specific activity of fatty acids and of cholesterol in mammary glands is far greater than in liver.

Further experiments convinced the authors that acetate, starch and glucose were equally effective as precursors of fatty acids and of cholesterol. (The lack of extensive data and the absence of statistical treatment leave the reader unconvinced on this one point.) Starch and glucose, however, seem to give rise to larger amounts of lactose and glycerol than does acetate. Since the simultaneous administration of labeled glucose and para-aminobenzoic acid led to the urinary excretion of labeled acetamidobenzoic acid, the authors became convinced that glucose is converted to acetate prior to the synthesis of fatty acids and of cholesterol. During the six hours following administration of labeled compounds, 25 per cent or more of the short-chain fatty acids were synthesized from acetate or from carbohydrate. The synthesis of long-chain fatty acids from these sources was much less. This lent support to earlier suggestions that the short-chain fatty acids are usually synthesized from simple precursors rather than resulting from the degradation of long-chain acids.

The low concentrations of some trace metals found in milk have made this food useful in the experimental production of anemia, although other effects of trace metal deficiency have not been so widely investigated. The role of iron, copper and manganese (added to milk) in the maintenance of liver catalase in the mouse has been studied by D. H. Adams (*Biochem. J.* **54,** *328 (1953)*). It had previously been reported that the livers of male mice show a higher level of this iron-containing enzyme than do females. Castration of the male lowers the enzyme level to slightly below that observed in females. Normal activity in the castrate is restored by injection of testosterone. Similarly, adrenocortical hormone is necessary for the maintenance of liver catalase levels.

In the present study, weanling albino mice were supplied with a ration consisting of homogenized whole milk, from a commercial source, supplemented with 3 per cent glucose. Iron, copper and manganese were added as needed, iron at a level of 50 micrograms, copper 10 micrograms, and manganese 5 micrograms per mouse per day. In one experiment, the development of liver catalase activity was studied using both a commercial stock ration and the milk-glucose diet supplemented with iron, copper and manganese. At weaning, liver catalase in the male was only slightly higher than in the female. Thereafter, activity in the male liver increased rapidly, while that in the female showed only a moderate increase. The two rations were approximately equally effective in supporting the development of liver catalase. In animals fed only the milk-glucose combination without trace-metal supplementation, liver catalase remained at the weaning level during an initial two-week period. Thereafter, the level increased sharply in both males and females, until after five weeks on the diet it reached the normal male level. During this time blood hemoglobin had fallen to 4 g. per cent. When the animals were continued on the unsupplemented ration, death ensued rapidly. If, on the other hand, they were transferred to a commercial stock ration, the hemoglobin levels rose, while the liver catalase of the females dropped precipitously toward the normal female level. The catalase levels in male livers remained essentially unchanged. After five weeks on the milk-glucose unsupplemented ration, the livers were cream colored and the animals showed large bald areas of skin.

THE LIPOTROPIC EFFECT OF THREONINE

Dietary factors other than choline are known to exert a lipotropic effect, that is, to prevent the accumulation of fat other than in the normal storage depots of the body. Protein was shown to be lipotropic several years ago. However, early workers felt that this effect was due chiefly to the methionine present (*see Nutrition Reviews* **11,** *321 (1953)*). H. C. Eckstein (*J. Biol. Chem.* **195,** *167 (1952)*) reinvestigated the possible lipotropic effect of various amino acids. The basal diet which was fed to rats contained 4.455 per cent of an amino acid mixture. The diet contained no choline. On this diet the rats developed fatty livers and the effects of single amino acid supplements were tested. Only methionine exerted a lipotropic effect; threonine and the other amino acids tested were not effective. Earlier, S. A. Singal, V. P. Sydenstricker, and J. M. Littlejohn (*Fed. Proc.* **8,** *251 (1949)*) had reported a lipotropic effect for threonine. These workers fed rats a basal diet which contained 9 per cent casein and 0.2 per cent choline. The average liver lipid content of rats fed this basal diet was 16 per cent. Supplementation of this diet with threonine reduced the average liver lipid concentration to 5.1 per cent.

These apparent discrepancies made it desirable to secure more information on the effect of threonine on liver fat deposition. The major difference in the basal diets employed by Eckstein and Singal *et al.* was the choline content. The basal diet employed by the latter workers contained 0.2 per cent choline whereas the basal diet employed by Eckstein contained no choline. It then appeared possible that the effect of threonine on fat deposition was modified by the composition of the basal diet. This suggestion received support from the data reported by A. E. Harper, W. J. Monson, D. A. Benton, and C. A. Elvehjem (*J. Nutrition* **50,** *383 (1953)*). These workers fed rats a basal diet containing 9.0 per cent casein, 0.13 per cent choline, and the usual vitamins, including folacin and vitamin B_{12}. As would be expected, animals receiving this basal diet developed fatty livers. When the basal diet was supplemented with 0.18 per cent threonine a marked lipotropic effect was observed. When this same basal diet was fed without choline, threonine exerted no lipotropic effect. These observations then reconcile the apparent discrepancies of earlier reports and show that threonine exerts a lipotropic effect only when the diet contains some choline.

Additional data concerning the lipotropic effect of threonine have been presented by A. E. Harper and co-workers (*J. Biol. Chem.* **206,** *151 (1954)*). These workers fed weanling Sprague-Dawley rats a basal diet containing 9.0 per cent casein, 0.3 per cent methionine, 5.0 per cent corn oil and 0.13 per cent choline. The diet contained the usual vitamins including folacin and vitamin B_{12}. The carbohydrate source was sucrose. This basal diet and the diet with various supplements were fed for two weeks, the animals were killed and the level of liver fat was determined. There were 6 animals per group.

In the first experiments Harper and coworkers studied the effects of supplementary gelatin, threonine and tryptophan on liver fat of rats receiving the basal diet. The average fat content of livers from rats fed the basal diet was 41.8 per cent on a dry weight basis. Supplementation of this diet with 6 per cent gelatin reduced the liver fat to 17.5 per cent. No effect on liver fat levels resulted from tryptophan supplementation at a level of 0.5 or 1 per cent. Since tryptophan did improve growth of the animals, the basal diet for subsequent experiments was modified by the addition of 1 per cent of this amino acid. The addition of 0.18 per cent threonine to the basal diet reduced liver fat to 25.3 per cent and supplementa-

tion with 0.36 per cent threonine resulted in a further reduction to 19 per cent. When the diet containing 0.36 per cent threonine was supplemented with an essential amino acid mixture equivalent to that supplied by 6 per cent gelatin no further reduction in liver fat was observed. Thus the lipotropic effect of 6 per cent gelatin could be duplicated by 0.36 per cent threonine.

The next experiments were designed to study the effects of glycine, choline and betaine on liver fat deposition and their relationship to the threonine effect. Supplementation of the basal diet with 1.5 per cent glycine, 1 per cent choline, or 1 per cent betaine reduced liver fat somewhat, but not to the same extent as did a supplement of 0.25 per cent threonine. Also, threonine exerted a lipotropic effect when added to the diet containing 1 per cent choline or betaine. When 1 per cent betaine or 1.5 per cent glycine was added to the diet containing threonine, liver fat was only slightly reduced below the level obtained with threonine alone. Thus it is possible that under the conditions employed glycine and betaine were effective because they spared threonine in some fashion.

When the basal diet was fed without choline the average liver fat content was 45.1 per cent. The addition of 0.36 per cent threonine to this diet resulted in an average liver fat content of 45.5 per cent. Again it was demonstrated that threonine exerted a lipotropic effect only when choline was present in the diet.

The final experiments were designed to study the effect of dietary fat on liver fat deposition and on the lipotropic effect of threonine. The basal diet was modified to be fat-free or to contain 10 per cent and 20 per cent corn oil and 5 per cent and 20 per cent butterfat in various experiments. These diets were fed with and without a supplement of 0.36 per cent threonine. The content of fat in the diet had no consistent effect on the content of fat in the liver. The lipotropic effect of threonine was also observed on all diets and appeared to be unrelated to the level of dietary fat.

These experiments clearly demonstrate a lipotropic effect for threonine. Also, it is clear that this effect is dependent on the presence of choline in the diet. The mechanism of the threonine effect is not known. It may be related to the synthesis of enzymes which function in phospholipid synthesis or in fatty acid oxidation.

THE PROTEIN-SPARING EFFECT OF DIETARY ANTIBIOTICS

The growth-promoting effect of antibiotics is well established, and a considerable body of data indicates that the effect is related to alterations in the intestinal flora of the animals. Also, the composition of the basal diet is known to influence the growth response to antibiotics; this has been most clearly established in the case of several of the B-vitamins (*Nutrition Reviews* **10,** *107* (*1952*)). It should be recalled that the early experiments by E. L. R. Stokstad *et al.* (*J. Biol. Chem.* **180,** *647* (*1949*)) which demonstrated a growth response of chicks to *S. aureofaciens* mash were designed to study the animal protein factor. The basal diets employed in these studies contained plant proteins and were deficient in several amino acids. This suggested the possibility that the growth-promoting effect of antibiotics was related to the protein of the basal diet.

The data reported by P. K. Vijayaraghavan, E. A. Murphy, and M. S. Dunn (*Arch. Biochem. Biophys.* **36,** *127* (*1952*)) supported such a suggestion. These workers found that mice fed a basal diet containing soybean meal or cottonseed meal exhibited a growth response to aureomycin. In contrast, when the basal diet contained casein or peanut meal the mice did not respond to dietary aureomycin. Since these workers did not

study the effects of individual amino acid supplements the results did not unequivocally relate the growth-promoting effect of antibiotics to the amino acid composition of the basal diet. The possibility remained that casein or peanut meal contained trace amounts of some unidentified nutrient which was in turn related to the growth-promoting effects of antibiotics much as was known to be the case with several of the identified vitamins.

The recent report of H. E. Sauberlich (*Antibiotics and Chemotherapy* **4**, *48 (1954)*) sheds considerable light on this subject and shows quite clearly that the growth-promoting effect of antibiotics is related to the amino acid composition of the basal diet. Sauberlich studied the growth response of weanling Sprague-Dawley rats to various antibiotics and the influence of dietary amino acids on this response.

In the first experiments the basal diet contained 95.5 per cent ground corn grain, which was 7.5 per cent protein, supplemented with 0.1 per cent DL-tryptophan, 0.5 per cent L-lysine hydrochloride, and the usual vitamins, including folacin and vitamin B_{12}. Growth was quite poor on this basal diet and was markedly improved by the addition of 0.6 per cent isoleucine, 0.2 per cent valine, and 0.2 per cent threonine. The addition of chlortetracycline or penicillin to the basal diet or to the amino acid-supplemented diet resulted in a considerable growth response. Since there was a growth response to either isoleucine, valine, and threonine or to antibiotics, it would seem that the antibiotics were sparing these amino acids.

The next experiments were designed to study the relationship of dietary antibiotics to tryptophan. The basal diet contained 85 per cent ground corn supplemented with cystine, lysine, threonine, valine, isoleucine and 0.025 per cent tryptophan. The addition of 0.075 per cent tryptophan to this diet approximately doubled the growth rate of the rats, thus indicating that tryptophan was limiting. When this tryptophan-deficient diet was supplemented with various antibiotics there was a growth response. These results then indicated that dietary antibiotics were also sparing tryptophan in some manner.

The relationship of antibiotics to lysine was observed when rats were given a basal diet containing 22.5 per cent sesame meal supplemented with methionine, histidine, cystine, isoleucine and 0.2 per cent lysine. The average four-week weight gain per rat on this basal diet was 55 g. The addition of 100 mg. of chlortetracycline per kilogram of diet increased growth to 89 g. while the addition of 0.3 per cent L-lysine hydrochloride increased growth to 82 g. When the lysine and chlortetracycline were added in combination the average four-week gain was 90 g. Thus the antibiotic completely replaced this portion of the lysine requirement.

In other experiments it was shown that when the protein content of the basal diet was increased the growth response to chlortetracycline was reduced or eliminated. When the diet contained 30 per cent peanut meal there was a marked growth response to the antibiotic whereas with 42 per cent peanut meal the addition of the antibiotic resulted in no further growth stimulation. Similar results were obtained with 8 per cent versus 20 per cent casein in the basal diet.

The results of these experiments indicate that dietary antibiotics spare the amino acid requirements of rats. The mechanism of this sparing action is not known. Several lines of evidence indicate that the antibiotics act by altering the intestinal flora of the host animal. It is possible that in the presence of antibiotics the intestinal flora is so altered as to result in an increased synthesis of the essential amino acids. It is known that bacteria in the rumen of sheep can synthesize significant quantities of all the essential amino acids (J. K. Loosli *et al.*, *Science* **110**, *144 (1949)*), and this synthesis is presumed to be the mechanism whereby these animals

utilize nonprotein nitrogen. It would be of interest to investigate the utilization of nonprotein nitrogen by rats fed antibiotics.

The results of these and other similar experiments may be of considerable practical importance. Protein is the most expensive dietary item for the livestock producer and estimates of the world's food supply indicate that protein is the limiting factor. Thus any mechanism for extending the protein supply is of considerable economic and sociologic importance.

ALTERNATIVE METABOLIC PATHWAYS FOR FRUCTOSE IN THE LIVER

At least two distinct pathways exist for the metabolism of fructose-1-phosphate in the liver, a rapid catabolic and a slower "assimilative" one. Liver fructokinase catalyzes the phosphorylation of fructose by ATP, yielding fructose-1-phosphate (M. W. Slein, G. T. Cori, and C. F. Cori, *J. Biol. Chem.* **186,** *763 (1950)*; F. Leuthardt and E. Testa, *Helvet. chim. acta* **34,** *931 (1951)*).

The direct catabolism of fructose-1-phosphate has been demonstrated by H. G. Hers, T. Kusaka, and C. De Duve (*Second International Congress Biochem., p. 291, Paris (1952)*). These workers found that fructose-1-phosphate could be broken down directly to glyceraldehyde and dihydroxyacetone phosphate, without prior phosphorylation to fructose-1,6-diphosphate. They believed that aldolase catalyzed this breakdown. It is probable that glyceraldehyde phosphate is formed from the glyceraldehyde resulting from this breakdown of fructose-1-phosphate, glyceraldehydekinase and ATP being involved in this conversion. The triosephosphates may then follow the conventional Embden-Myerhof scheme.

F. E. Leuthardt, E. Testa, and H. P. Wolf (*Tagg. d. Schweiz. Physiol. u. Pharm., Genf, 25 (1952)*), also studying the direct catabolism of fructose-1-phosphate, found that purified aldolase was without catalytic effect on this breakdown. The enzyme which catalyzed the conversion of fructose-1-phosphate to dihydroxyacetone phosphate and glyceraldehyde, was named 1-phosphofructaldolase by Leuthardt.

E. Helmreich, S. Goldschmidt, W. Lamprecht, and F. Ritzl (*Z. f. physiol. Chem.* **292,** *184 (1953)*) have added considerable experimental evidence for a direct breakdown of fructose-1-phosphate. This group infused intact male rats with solutions of D-fructose, D-glucose, D-galactose, L-sorbose, and D-mannose containing sulfanilamide and measured the amount of acetylsulfanilamide formed by the livers of these animals ten minutes after receiving the infusion. The amount of acetylsulfanilamide is a measure of the "active acetate" (acetyl CoA) formed (N. O. Kaplan and F. Lipmann, *J. Biol. Chem.* **174,** *37 (1948)*). Of the sugars tested only fructose produced a significant increase in "active acetate" above the control value obtained when sulfanilamide and sodium chloride were infused. Corresponding to an increased formation of "active acetate," ten minutes after supply of fructose there was also an increased formation of pyruvic acid which reached its maximum concentration at this time. In contrast, the increased formation of pyruvic acid after supply of glucose reached its maximum concentration only after thirty minutes. The extra pyruvic acid formed in the first ten minutes after fructose infusion was largely converted to "active acetate." Helmreich *et al.* concluded that when large amounts of fructose are supplied to the liver, the fructose is broken down preferentially first to triosephosphate by means of 1-phosphofructaldolase.

The slower "assimilative" pathway of fructose metabolism has been described by G. T. Cori, S. Ochoa, M. W. Slein, and C. F. Cori (*Biochim. et biophys. acta* **7,** *304 (1951)*). They isolated a protein fraction from rat liver which catalyzed the conversion of fructose-1-phosphate to glucose-6-phosphate via an unknown intermediate. The enzyme

responsible for this conversion, termed phosphofructomutase, was not found in muscle or brain tissue. They also found that the concentration of 6-phosphofructokinase, the enzyme which catalyzes the conversion of fructose-6-phosphate to fructose-1,6-diphosphate (Embden-Myerhof scheme), was relatively low in the liver. They stated, "This favors the conversion of hexose-6-phosphate to glycogen or to glucose. As is well known, fructose is a good glycogen former in the liver of normal animals and gives rise to extra glucose in the diabetic animal."

It seems likely from the above evidence that fructose follows at least one metabolic pathway in the liver which is independent of glucose metabolism and that this pathway accounts for the major catabolism of fructose. In the diabetic liver, therefore, fructose can be broken down into triosephosphate without the aid of any of the enzymes involved in the breakdown of glucose to triosephosphate. For this reason the argument for inhibition at the glucokinase (hexokinase) level in the diabetic liver based on different oxidation rates of glucose and fructose no longer seems tenable.

ASCORBIC ACID SYNTHESIS IN ANIMALS

In a recent series of publications C. G. King and co-workers have elucidated the major steps in the synthesis of ascorbic acid in the rat, an animal which, like the majority of mammals, does not require an extrinsic source of this dietary essential.

The central compound in this biosynthesis is glucuronic acid. A constituent of many polysaccharides in plants, bacteria and animals, it has often been considered essentially a protective agent, important in many "detoxification" reactions. Its chemical structure resembles that of ascorbic acid. Early studies (H. E. Longenecker, R. R. Musulin, R. H. Tully, and C. G. King, *J. Biol. Chem.* **129,** *445* (*1939*)) which demonstrated that a number of substances, in particular chloretone, caused a simultaneous increase in the urinary secretion of both ascorbic acid and glucuronic acid, hinted at a physiologic relationship.

Glucuronic acid in turn has been shown to arise from glycogen and hence glucose (F. Schmid, *Compt. rend. Soc. de biol.* **123,** *223* (*1936*)), as well as glycogenic amino acids. The likelihood of the role of glucuronic acid as a precursor of ascorbic acid, already suggested by earlier work, received considerable additional support when King and his colleagues (S. S. Jackel, E. H. Mosbach, and C. G. King, *Arch. Biochem. Biophys.* **31,** *442* (*1951*); H. H. Horowitz, A. P. Doerschuk, and King, *J. Biol. Chem.* **199,** *193* (*1952*); Horowitz and King, *Ibid.* **200,** *125* (*1953*)) demonstrated that C^{14}-labeled glucose is converted to ascorbic acid in the albino rat without chain fragmentation or change in steric configuration around carbons 2 and 3 of glucose (carbons 4 and 5 of ascorbic acid). The metabolism of glucuronic acid itself has received considerable attention. J. F. Douglas and King (*Ibid.* **198,** *187* (*1952*)) had shown that when uniformly labeled D-glucuronic acid-C^{14} is administered to normal and to borneol-fed guinea pigs, approximately one fourth of the C^{14} given is recovered in respiratory carbon dioxide within three hours, and almost one-half appears in the urine within twenty-four hours; only very small amounts remain in specific tissues. Part of the C^{14} is excreted in the urine as bornyl glucuronide, which indicates that at least a portion of the glucuronic acid is used in forming the conjugate.

In a subsequent study J. F. Douglas and C. G. King (*J. Biol. Chem.* **203,** *889* (*1953*)) demonstrated by means of complete degradation of the urinary glucuronide that glucuronic acid is at least partially cleaved to one or more 3-carbon fragments. Examination of the end products of glucurone-6-C^{14} in borneol-fed guinea pigs indicated that

exogenous glucuronic acid was not utilized in appreciable quantities for direct conjugation, but was metabolized largely to expired carbon dioxide.

When uniformly labeled glucuronic acid was administered to albino rats it was found that the compound was also largely (and rapidly) converted to carbon dioxide, 46 per cent of the initial dose being recovered in expired carbon dioxide within twenty-four hours. An approximately equal amount of C^{14} was found in the urine while only small amounts of C^{14} could be detected in tissues.

On the basis of this extensive knowledge of the metabolic behavior of glucuronic acid, H. H. Horowitz and C. G. King (*J. Biol. Chem.* **205,** *815 (1953)*) have been able to complete the scheme of biosynthesis of ascorbic acid from glucose to glucuronic acid to ascorbic acid. Rats received chloretone until their ascorbic acid excretion was stabilized at a high daily level (30 mg. per day). They were then injected with uniformly labeled glucuronic acid (given as the lactone). The urinary ascorbic acid was isolated and crystallized, and purified to a high degree so as to remove any trace of unmetabolized C^{14}-labeled glucuronolactone. Constant isotope activity was reached in the isolated ascorbic acid. Demonstration of the purity of the labeled ascorbic acid was obtained by recrystallization, cleavage of the isolated product, and paper chromatography. All these tests indicated that the C^{14} activity of the urinary ascorbic acid could not be due to contamination. The radioactivity of each carbon of ascorbic acid was determined, found to be constant from animal to animal and consistent with the hypothesis that no chain fragmentation occurred in the conversion of glucurone to ascorbic acid. The results thus obtained demonstrated not only that glucuronic acid is a direct precursor of ascorbic acid but also that glucuronic acid is closer than glucose to ascorbic acid along its route of synthesis. Horowitz and King, on the basis of these experiments, suggested a one-step, acid-base type intramolecular oxidation-reduction and enolization as follows: D-glucuronic acid-gamma-lactone → 2 keto-L-gulono-gamma-lactone → L-ascorbic acid.

FLUORINE: ESSENTIAL NUTRIENT?

In recent years, the question of whether fluorine is an essential nutrient has been debated widely. No final answer to this question is available. One of the principal reasons for the inability to formulate an answer has been that experimental diets composed of either natural foodstuffs or purified ingredients have without exception contained appreciable amounts of fluorides which were extremely difficult, if not impossible, to remove completely. In order to obviate the present necessity to purify each dietary component, the use of water culture for the growth of plants as sources of fluoride-free dietary components has been proposed and tested on a small scale by J. F. McClendon (*Fed. Proc.* **3,** *94 (1944)*). He believed that nutrient solutions which would support plant growth could be produced from fluoride-free reagent-grade chemicals and that these plants would be fluoride-free. The various components of these plants could then be used for the preparation of a more nearly fluoride-deficient ration than has heretofore been possible.

Recently the results of a larger scale experimental project based on water culture of the dietary components have been described by J. F. McClendon and J. Gershon-Cohen (*J. Agr. Food Chem.* **1,** *464 (1953)*). In addition to the development of fluoride-free diets, the method was also used for the preparation of diets which were deficient in iodine and sodium. The water used for the nutrient solutions was rain water collected at their experiment station, twenty-five miles from Philadelphia. Analyses of this water indicated a fluoride content of 0.002 to 0.004

parts per million (p.p.m.) which was exceedingly low in view of the potential contamination with fluorides from sea spray, coal smoke, or open-hearth furnaces. Wooden tanks which were lined with aluminum foil and coated with asphalt varnish were used to hold the nutrient solutions. The seeds were planted on aluminum foil laid over wire mesh with a wick of the previous year's stalks to draw up the nutrient solution. Frequent analyses of the nutrient solutions were made to determine the rate at which the minerals were used up by the plant crop. Iron and manganese were added daily in the ratio of 2 parts of iron to 1 part of manganese; the other salts were added once a month. Sufficient zinc and copper were present as impurities in the salt mixture. The best crop yields were obtained with sunflowers; corn was not always well pollinated.

The basal diet for the rats was composed of yellow corn, 30 per cent; sunflower seed, 30 per cent; sunflower leaves, 20 per cent; yeast, 10 per cent; and sucrose, 10 per cent. Since halogen-free yeast was unavailable commercially, the investigators prepared their own by a procedure which was described in detail. In order to use the above diet in a fluoride test, it was supplemented with adequate amounts of sodium and iodine. In addition, the control rats received drinking water which contained 20 p.p.m. of fluoride. It is noteworthy that the diet consumed by the control rats was prepared from field-grown crops rather than from water culture crops. This is an obvious departure from sound experimental procedure. The rats in this experiment were littermates, 21 days of age. After two months on these different diets, the rats were killed and histologic, radiographic and chemical studies were made of the teeth and bones. A similar experiment was conducted in which the basal diet was fortified with sodium and fluoride so that iodine was the only essential element known to be missing. Again the controls received a diet composed of field-grown crops.

Nineteen rats on the fluorine-free diet gained an average 11.2 g. during the two months as compared to 86.9 g. gained by the controls. The rats either failed to grow or grew at subnormal rates with diets deficient in either fluorine or iodine. The extremely slow growth of the control rats was not commented upon. The average gain of 10 g. per week among the controls is so far below the usual growth rate of 85 g. per week that these rats must have been deficient in one or more nutritional essentials. Most of the molar teeth in the rats on the fluorine-free diet were carious, with 10.2 carious teeth per rat as compared with 0.5 carious teeth for each control rat. The thyroid glands of the control rats averaged 8 mg. per 100 g. of body weight, but on the iodine-free diet the goitrous thyroids weighed 18 to 42 mg. per 100 g. of body weight.

Unquestionably, great difficulties attend efforts to extract any single mineral or vitamin from a natural food substance. There is a definite potentiality that water culture may make possible the production of such a limited diet that sound studies of the essentiality of fluorine or any other trace element can now be conducted. The growth of a foodstuff devoid of any element must depend on whether that element is essential to the plant. It has been claimed that iodine stimulates the growth of certain plants and it is thought that sodium may replace part of the potassium in other plants. These studies have suggested that sodium or halogens were not essential for the crops grown; on the basis of the presented evidence, there are insufficient grounds to make the unqualified statement that they are not essential for these crops.

At the opposite end of the spectrum from the phase of fluoride ingestion described above is a recent paper by R. J. H. Mick (*Dent. Items of Interest* **75**, *566 (1953)*). Data were presented which have been interpreted by the author to indicate toxic manifestations from the consumption by rats of drinking water which was variously stated within the same paragraph to contain "2.0 ppm. of fluorine" and "2.0 ppm. of sodium fluoride." It is noteworthy that the com-

position of the stock ration was not given; furthermore, there was no comment on the fluoride content of this ration. The results were obtained from several groups of rats which were described with a similar confusion of statements and with absence of many of the details needed for an accurate appraisal of the data. Presumably all rats involved were the offspring of 6 young adult female rats and 2 young adult males which were representatives of the Wistar strain. For the first two months of the experiment, the rats were given drinking water containing "0.4 ppm. of fluorine" and thereafter the higher level of fluoride described above. In all, six litters of rats were born. Fluoride analyses were made on four separate series of rats. Two series of rats each were maintained at a 2.0 p.p.m. level for the first four weeks of life and killed at 3½ months of age. Another series of 11 rats was fed this level of fluoride for eight weeks at which time they were killed. Three of the breeding females comprised the fourth series of animals which were killed after three months of waterborne fluoride ingestion and a final month without added fluoride.

The highest fluoride analyses were observed in the teeth and bones of the last series with values of 175 and 220 p.p.m. respectively. The previous series had appreciably lower levels.

Among the offspring some abnormalities were reported. The incisor teeth were thicker than normal with flat broad surfaces on the biting edges and the pulp chamber exposed. The posterior teeth were said to be not as firmly fixed in the maxilla as in normal rats and the roots were more exposed. The dental arches were more constricted and the teeth were free of pigment, chalky and hypocalcified. The general rate of growth of the rats fed the fluoride solution was stated without supporting facts to be less than that of animals fed the same ration without added fluorides.

In the discussion of his results in relation to other published data, Mick (*loc. cit.*) believed that the abnormalities observed compared closely with those produced by M. C. Smith, E. M. Lantz, and H. V. Smith (*Science* **74,** *244 (1931)*), where 1 per cent sodium fluoride was added to the diet. He adhered to this belief despite the fact that there was a several hundred-fold higher level of fluoride ingestion in the studies by Smith *et al.* Mick believed that this was due to the fact that his observations were made on second generation animals and that an increased sensitivity through the reproductive cycle was demonstrated thereby. However, it is noteworthy that the careful studies of G. J. Cox and co-workers (*J. Dent. Res.* **18,** *481 (1939)*) demonstrated the supplementation of their breeding ration with 10 to 40 p.p.m. of fluoride to be entirely compatible with normal reproduction and with the birth of normal offspring.

Since neither the composition of the stock ration nor its fluoride content is given, one can only speculate on the possible causes of the pathology observed. Certainly there is not a shred of evidence that the pathologic changes were due to the amount of fluoride added unless perchance there was some error in calculation or in weighing which accidentally provided much more fluoride than was believed to have been added. The possibility exists that there may have been some deficiency in the breeding ration which caused congenital abnormalities of the type described in rats by J. Warkany and C. B. Roth (*J. Nutrition* **35,** *1 (1948)*).

Certainly, on the basis of the studies by McClendon and Gershon-Cohen and those of Mick, we have no right to accept the essentiality of fluorides for rat growth nor the toxicity of comparable low amounts of fluorides in the second generation. Studies of both the above types lend themselves to misinterpretation and misrepresentation of the facts and may easily serve to obscure well-substantiated data obtained from more carefully planned and conducted experiments.

NOTES

Letters to the Editor

Dear Sir:

There are two errors which change the meaning of the review "*In Vitro* Studies of Vitamin A Formation" (*Nutrition Reviews* **12,** *23* (*1954*)), and might be worth correcting. On page 24, right hand column, line 16: "*in vitro*" should read "*in vivo.*" In the second paragraph, right hand column, line 15: "improved" should read "impaired."

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Dear Sir:

In reference to the article "Metabolism of Urea" which appeared in the January issue of *Nutrition Reviews* (**12,** *17* (*1954*)), I should like to draw your attention to the work done in Sheffield (Great Britain) by H. L. Kornberg, R. E. Davies, and D. R. Wood, reported in the Proceedings of the Biochemical Society (*Biochemical J.* **55,** *ii* (*1953*)). These authors have found that the $C^{14}O_2$ output in the expired air which is observed in cats after injection of (C^{14})-urea is due to the action of the urease-containing organisms, existing in the mouth, nasopharynx and esophagus of the animals. Even the gastric urease seems to be of bacterial origin in the cat (Kornberg, Davies, and Wood, *Ibid.* **55,** *iii* (*1953*)). The results of the British workers imply that urease does not play an essential role in the physiology of the stomach.

The conditions of the experiments are, of course, very different from those of the experiments reported in *Nutrition Reviews*, but it seems clear that the results of Kornberg and co-workers do not lend support to the existence of an enzymatic transformation of urea in the tissues of the cat which would explain the presence of $C^{14}O_2$ in the expired air.

The claim of Leifer and co-workers, and of Zbarsky and Wright, that urea can undergo metabolic activity in the animal body would be, if correct, of great importance for our understanding of nitrogen metabolism. However, in view of the results reported by the British authors, a careful reconsideration of the interpretation of the experimental results by Leifer and co-workers and Zbarsky and Wright is called for.

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Nutritive Value of Varieties of Grapefruit

Citrus fruits make an important contribution to the human diet because of their high content of ascorbic acid. Recent studies by M.-U.-D. Khan and G. Mackinney (*Plant Physiol.* **28,** *550* (*1953*)) on grapefruit indicate that some varieties of this fruit may also provide the consumer with appreciable quantities of beta-carotene. The Marsh White or colorless grapefruit of commerce mutated to produce Marsh Pink, with pink flesh. Bud sports of Marsh Pink gave rise to Ruby (Texas Pink and Arizona Red Blush) with deep pink flesh.

The fresh flesh of commercial samples of these fruits from California, Texas and Arizona were analyzed chromatographically and spectroscopically for carotenoids. Marsh White contained less than 0.1 parts per million (p.p.m.) of zeta-carotene and phytofluene and only a trace of beta-carotene, with no lycopene. The pink varieties all contained only very small amounts of the colorless carotenoids (less than 0.2 p.p.m.); however, appreciable quantities of beta-carotene appeared (from 1.3 to 2.0 p.p.m.) and lycopene was present to the extent of 0.3 to 2.4 p.p.m. In Marsh Pink beta-carotene predominated 4:1 over lycopene. In Texas Pink the lycopene content was slightly higher than beta-carotene, which was in turn higher than in any other variety.

The role of mutations in establishing the predominance of beta-carotene or lycopene (no vitamin A activity) is particularly evident in the varieties of pink grapefruit. Lack of knowledge concerning gene differences prevents application of this information to development of a mechanism of carotenoid synthesis. These data indicate a distinct nutritional advantage of the pink grapefruit varieties over the white varieties.

Folacin Losses in Cold-Storage Eggs

Since several million cases of shell eggs are stored during periods of high production and low prices, to be used later when economic conditions are different, any change in nutritional quality during storage is important. The poultry industry has put more effort into study of physical changes in eggs which affect their appearance than into the nature of chemical changes. Previous studies have indicated loss of the following during egg storage: protein, riboflavin, niacin, vitamin B_6, and pantothenic acid. Biotin and choline are quite stable. R. J. Evans, J. A. Davidson, D. Bauer, and H. A. Butts (*J. Agr. Food Chem.* **1,** *170 (1953)*) have studied the stability of folacin during storage.

Fresh raw eggs from 10 White Leghorn hens fed a standard diet were stored at 0°C. and assayed at three-, six-, and twelve-month intervals for folacin. Whites and yolks were analyzed separately. No loss occurred during the first three months. During the second three months, 16 per cent of folacin was lost and during twelve months 27 per cent was lost. The loss was entirely from the egg yolk, which contains almost nine-tenths of the folacin of the egg. Folacin apparently does not move as freely through the vitelline membrane as does pantothenic acid. Loss of water during storage complicates the expression of losses in various parts of the egg. Folacin concentration decreased in twelve months by only 21 per cent.

Lind Bicentenary Symposium

A recently issued number of the *Proceedings of the Nutrition Society* (Great Britain) is devoted to addresses made May 22 and 23, 1953, at the Bicentenary Symposium held at the University of Edinburgh in honor of James Lind, M. D., whose "Treatise of the Scurvy" was published in Edinburgh in 1753. A total of 15 papers is presented, mostly by British and American scientists, and they cover a wide range, but all deal with ascorbic acid.

Recent Nutrition Books

Five Year Cumulative Index to [*Nutrition Reviews.* Volumes 6–10, January 1948–December 1952. Prepared by Elmer H. Stotz and Christine S. Wilson. Published by The Nutrition Foundation, Inc., New York, N. Y., 1953. Pp. 134. Price: $1.50. Copies are available from The Nutrition Foundation, Inc., Chrysler Building, New York 17, N. Y.

Vitamin C Requirement of Human Adults. A Report by the Vitamin C Subcommittee of the Accessory Food Factors Committee and A. E. Barnes, W. Bartley, I. M. Frankau, G. A. Higgins, J. Pemberton, G. L. Roberts, and H. R. Vickers. Compiled by W. Bartley, H. A. Krebs, and J. R. P. O'Brien. Medical Research Council Special Report Series No. 280. Published by Her Majesty's Stationery Office, London, 1953. Pp. 179 + 14 plates. Price: 17s. 6d. net ($4.15).

School Feeding. Its Contribution to Child Nutrition. FAO Nutritional Studies No. 10. Marjorie L. Scott. Food and Agriculture Organization of the United Nations, Rome, Italy, 1953. Pp. 129. Price $1.00; 5 shillings.

Map—*Study in Human Starvation Sources of Selected Foods.* Prepared and published by the American Geographical Society, Broadway at 156th Street, New York 32, N. Y., April 1953. Price: $1.25 folded; $1.50 flat (in roll).